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Fort Detrick, Maryland 21702-5012

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14. ABSTRACT In this grant we propose to elucidate the signaling pathway that translates VEGFR activation into elevated vessel permeability, in endothelial cells within living breast tumor models. The working hypothesis is that the signaling pathway involved is a constitutively active form of the pathway shown for healthy mesenteric microvessels. Progress to date includes the recruitment of personnel to the new laboratory, the development and testing of a novel method for the measurement of convective flow in tumors in vivo, the investigation of second harmonic generation as a reproducible measure of photodamage in tissue during diffusion and permeability measurements, and preliminary experiments to evaluate methodology for tasks to commence in upcoming years.					
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Introduction.

Current anti-angiogenic therapies focus on the earliest steps in the angiogenic signaling cascades and try to prevent angiogenic molecules (i.e. VEGF, Ang-1, TGF- α) from reaching endothelial cells or try to prevent activation of their endothelial cell (EC) receptors. However, the study of downstream steps, within tumor ECs, as an avenue for treatment has been neglected. Furthermore, due to a lack of appropriate *in vivo* imaging and measurement tools, these EC signaling cascades have been explored almost exclusively in thin preparations of healthy vessels (i.e. vessels in the easily accessible, 25 micron mesenteric membrane) or in endothelial cells in a dish, and the signaling machinery that is delineated varies depending upon which type of healthy vessel provides the ECs. As tumor vessels are fundamentally unlike any of the healthy vessels in the body, we don't know which of the known signaling pathways are involved in *in vivo* tumor angiogenesis, *or if any of them are*. Consequently, we propose to elucidate the signaling pathway that translates VEGFR activation into elevated vessel permeability, in endothelial cells within living breast tumor models. The working hypothesis is that the signaling pathway involved is a constitutively active form of the pathway shown for healthy mesenteric microvessels. We have identified several signaling molecules that we hypothesize will play key roles in that pathway. In each case we will pharmacologically enhance or inhibit the action of a given molecule, and use advanced *in vivo* imaging techniques that we are currently developing or have previously developed to probe the resultant alterations in the VEGF/permeability relationship, with EC internal calcium dynamics as a key intermediate readout. Elucidation of this pathway is motivated by the desire to find new therapeutic targets, with which to block tumor angiogenesis and hence restrict tumor growth. Current angiogenic therapies, which favor blocking transit of an angiogenic factor to the ECs or inhibition of receptor activation, often fail because there can be several parallel pathways for angiogenic signals to travel from tumor cells to ECs, and when one is blocked, others are utilized. Signaling that occurs downstream of endothelial receptor activation may provide a signaling 'bottleneck' that several angiogenic factors utilize in common and hence may provide a uniquely powerful therapeutic target which circumvents the development of drug resistance.

Body

The Department of Defense BCRP generously allowed me to delay the start date on this grant until September first of last year, which was also the date I began to set up my new laboratory in the Department of Biomedical Engineering at the University of Rochester Medical Center. Having a stable source funding has allowed me to selectively recruit a top-notch group of personnel extremely rapidly, and has greatly accelerated the process of setting up a new lab in a new location. Consequently we have several interesting results to report. Chronologically, the earliest goals in my original Statement of Work are actually encompassed in tasks 7 and 9. In its entirety, Task 7 is:

Task 7. Determine the relative contribution of convection versus diffusion in transport of fluorescent tracer out of a tumor vessel. (Years 1 and 2)

A. Develop theory and perform *in vitro* tests of the ability of Multiphoton Fluorescence Recovery After Photobleaching (MPFRAP) to simultaneously measure diffusion and convection.

- B. Determine relative contribution of convection versus diffusion in transport out of a tumor vessel during steady state conditions.
- C. Determine relative contribution of convection versus diffusion in transport out of a tumor vessel after acute alteration in tumor vessel permeability.

My first accomplishment in pursuit of this Task was the recruitment of Kelley Dunbar, a first-year Ph.D. student from the Department of Physics. We have begun to test the novel theoretical formula which we believe describes MPFRAP in conditions of combined flow and diffusion (development and testing of this formula are the goal of Task 1a):

$$F(t) = F_0 \sum_{n=0}^{\infty} \frac{(-\beta)^n e^{-4n(vt/w)^2/(1+n+2nt/\tau)}}{n!(1+n+2nt/\tau)\sqrt{1+n+2nt/R\tau}} \quad (1)$$

where F_0 is the pre-bleach fluorescence, β is the bleach depth parameter, v is the convective flow in the r direction, w is the e^{-2} beam radius in the r direction, R is the square of the beam radii in the r and z directions, τ is the characteristic diffusion time given by $t=w^2/8D$ and D is the diffusion coefficient. This equation is for the specific case of the laser propagating along the z axis and flow in the radial direction. We also have the equations for the more general case of flow in an arbitrary direction. Testing of this equation has involved the construction and calibration of a controlled linear flow apparatus. As shown in figure 1 a syringe pump is connected to a large open-topped reservoir and the outflow is directly imaged with our custom-built multiphoton laser-scanning microscope (also constructed in the last year but paid for with university startup funds). A very dilute solution of $\sim 0.1 \mu\text{m}$ diameter fluorescently labeled polystyrene beads mixed with 2M MW FITC-Dextran is forced out of the tube at different linear velocities controlled by the syringe pump. Line scans are used to measure the flow velocity of fluorescent beads and this velocity measure is used as the gold standard to compare simultaneous MPFRAP measurements of diffusion and convection of the 2M MW FITC-Dextran. As shown in figure 2, preliminary data suggests that the derivations leading to equation 1 are correct, and it accurately describes MPFRAP with linear flow for certain combinations of tracer diffusion coefficient, linear velocity, and signal-to-noise ratio. What remains to be determined is the full range of velocities, diffusion coefficients, and signal-to-noise ratios over which this technique is valid, and if that range is a useful range for our specific biological questions.

Figure 1. Apparatus constructed to test the validity of equation 1. A dilute solution of fluorescent beads mixed in FITC-Dextran is injected into the chamber at a constant linear velocity. Linescan measurements of the velocity of the beads are used as the gold standard to compare with simultaneous MPFRAP measurements of diffusion and convection of the FITC-Dextran.

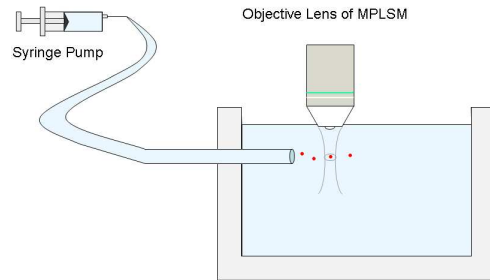
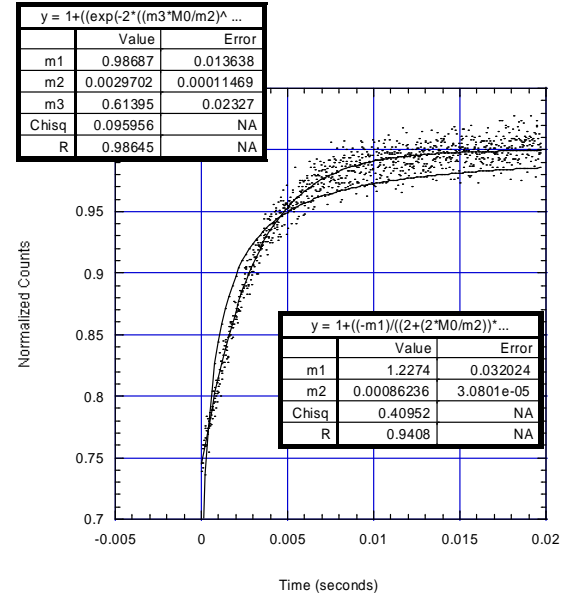
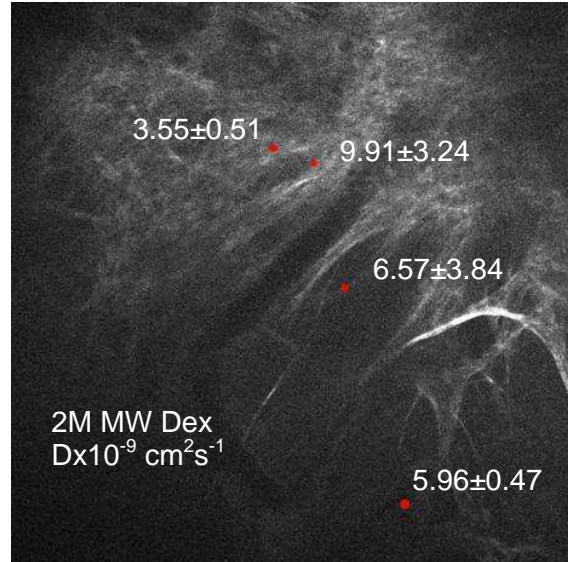


Figure 2. One of several results of the testing of equation 1. A known flow velocity of 140 microns/sec was introduced in a solution of 2M MW FITC-Dextran ($D=2 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$) and MPFRAP was performed. A fit was generated, producing a measured D of $1.910^{-7} \text{ cm}^2 \text{ s}^{-1}$ and a velocity of 140 microns/sec. Note the second (poorer) fit line, generated using only pure diffusion analysis.



Application of this new modification of the MPFRAP technique to the difficult *in vivo* environment of the living tumor is the goal of Tasks 1B and 1C. In pursuit of this goal we have begun to perform MPFRAP in tumors *in vivo*. Note that MPFRAP in tumors *in vivo* has not yet been published to our knowledge, and will in itself represent a significant landmark almost equal to my original development of the MPFRAP technique in 1999 [1]. *In vivo* MPFRAP is a technically challenging task primarily because the parked beam required for MPFRAP experiments is expected to generate some amount of photodamage, and it is vitally important to quantify, or at least set an upper bound on, the effects that this photodamage has on the sample and on the results of the diffusion/convection measurements. Consequently, implicit in Tasks 1b and c (the use of MPFRAP *in vivo*) is the establishment of a method to quantify or set an upper bound on the effects of photodamage during an *in vivo* MPFRAP experiment. Interestingly, in an earlier paper I showed that the global second harmonic generation (SHG) signal from the tumor extracellular matrix is a valid measure of the total collagen content of that tumor, which itself predicts the average macromolecular diffusion coefficient in the tumor [2]. We therefore hypothesized that the *local* second harmonic signal from the tumor extracellular matrix will provide a reproducible measure of local photodamage. We are currently testing this hypothesis, and have been investigating the variability in the local SHG signal from extracellular matrix, and its relationship to the local diffusion in tumors as measured with MPFRAP. To this point in time, the local SHG signal and the local diffusion coefficient measured with *in vivo* MPFRAP do not show a statistically significant correlation (see figure 3), presumably due to the large intrinsic variability in each parameter throughout the tumor. This high intrinsic variability suggests that local SHG may not be a good measure of local diffusion-altering photodamage.

Figure 3. Results of a series of MPFRAP experiments in an MCaIV murine mammary adenocarcinoma growing in the dorsal skinfold chamber of a SCID mouse. Each measurement was a series of 10 individual MPFRAP measurements (using only D and bleach depth as free parameters) performed at the nearby red dot. There is no statistically significant correlation between the local diffusion measure and the local second harmonic intensity.



The other Task in the original Statement of Work which is intended to commence in year one is Task 9, which in its entirety is:

Task 9. Establish a reproducible measure of photodamage during a permeability measurement. (Years 1 and 2)

- A.** Evaluate systematic alterations in the fluorescence-versus-time curve as a reproducible measure of photodamage during permeability measurements.
- B.** Evaluate successive permeability measurements with distinct markers as a reproducible measure of photodamage during permeability measurements.
- C.** Evaluate second harmonic imaging of the adjacent matrix as a reproducible measure of photodamage during permeability measurements.

In pursuit of this Task I have recruited Xiaoxing Han, a first year graduate student from the Institute of Optics, although he will not have to draw salary directly from the Era of Hope Scholar Award until later this summer. Similar to Task 7, we hypothesize that a reproducible measure of local photodamage due to permeability measurements is provided by the local second harmonic generation signal from the extracellular matrix (hence the formulation of Task 9C). In pursuit of this Task, Xiaoxing has been learning how to image tumors with SHG and is constructing a detection scheme that will interrogate both the forward- and backwards-scattered SHG signal from tumors in our animal models, whose ratio provides detailed structural information about the collagen fibers [3] and which may provide a better measure of photodamage than simple SHG intensity

The other Tasks in the original Statement of Work are intended to start in year 2 or later, but we have already made substantive progress on several of them. Specifically, I hired Kelley Madden, a Research Assistant Professor, and Ryan Burke, a first year BME student (whose salary will not appear on the grant until this fall as BME students are free for the first 15 months). They have been developing the molecular biology abilities of our

new laboratory, have been honing their abilities on our complex custom-built multiphoton laser-scanning microscope, and, most importantly, have been testing our future *in vivo* tumor endothelial cell imaging protocols on bovine aortic endothelial cells (BAECs) in culture. Figure 4a is an image they generated of Fura-2-AM-loaded BAECs and figure 4b is a fluorescence-versus-time curve of several endothelial cells showing a stereotypical response to bath application of VEGF. These calcium imaging techniques, when applied *in vivo* to tumor endothelial cells, will be pivotal in the upcoming tasks 1-6, scheduled to start in year 3.

Figure 4a. Image of Fura-2-AM-loaded bovine aortic endothelial cells. The fluorescence intensity of Fura-2 scales inversely with the cytoplasmic calcium concentration. These cells were imaged on our new custom built multiphoton laser-scanning microscope. They were excited at 800nm and imaged with a 580DF150 emission filter.

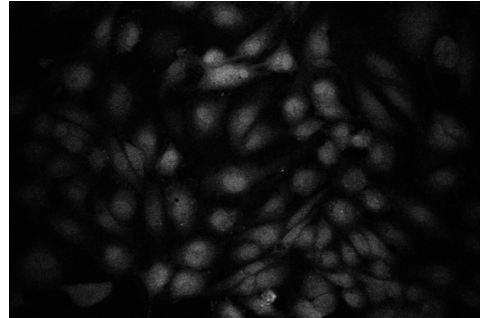
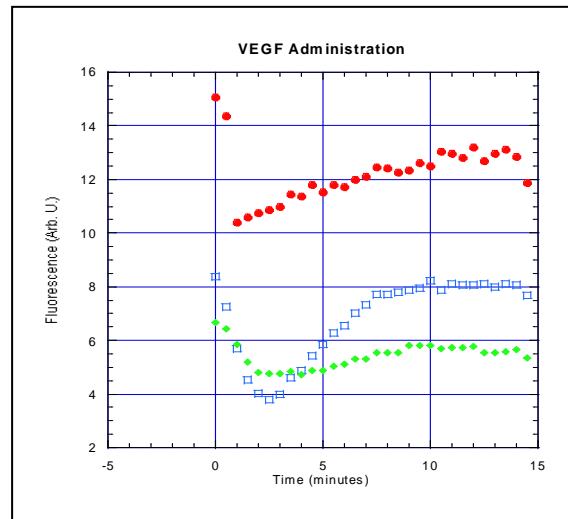


Figure 4b. Timecourse of fluorescence signal from several Fura-loaded endothelial cells *in vitro*, after bath application of VEGF at $t=0$. Note that Fura-2 fluorescence decreases upon binding to calcium, hence these curves display the stereotypical elevation in cytoplasmic calcium produced by acute application of VEGF.



In addition to first validating our techniques *in vitro*, in order to work out all the kinks before we move to the more challenging *in vivo* situation, we have also performed some preliminary *in vivo* work. Specifically we have performed pilot studies for Task 1a (scheduled to commence in Year 3). Task 1 in its entirety is:

Task 1. Determine role of external calcium influx on translation of VEGFR2 activation to tumor endothelial cell (TEC) calcium signals and tumor vessel permeability. (Year 3)

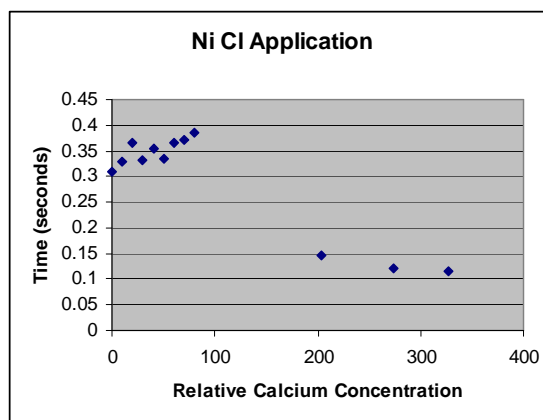
A. Apply NiCl (blocks plasmalemmal calcium channels) and CaCl_2 to a tumor vessel via pipette and observe the TEC calcium response and subsequent permeability change.

B. Elevate TEC calcium and vascular permeability with extrinsic VEGF then attempt to block this elevation by repeating with NiCl.

C. Reduce TEC calcium and vascular permeability with VEGF blockade and attempt to recover baseline calcium and permeability with CaCl_2 .

To perform a pilot study evaluating the methodology of Task 1a, we imaged tumor endothelial cells in TG1-1 mammary adenocarcinomas growing in the dorsal skinfold chamber of TIE-2 GFP transgenic mice. In these tumors, the endothelial cells are highlighted with green fluorescent protein. We applied Rhod-2-AM to the tumor surface which then loaded most cells in the field of view with a cytoplasmic indicator dye (Rhod-2) whose fluorescence increases with increasing calcium concentration. Note that Fura-2 could not be used because of spectral overlap with GFP. Then we applied NiCl_2 to the tumor surface and imaged the resultant change in Rhod-2 fluorescence, using the GFP signal as a marker for tumor endothelial cells. As shown in figure 5, the average tumor endothelial cell calcium concentration rapidly decreased after NiCl_2 application in a statistically significant manner, suggesting a steady-state influx of calcium ions from the extracellular space. Note that the data is presented as the fluorescence of Rhod2 divided by the fluorescence of GFP, to normalize away any shifts in the focal plane.

Figure 5. Effect of superficial NiCl_2 application on the cytoplasmic calcium concentration of endothelial cells growing within the outer ~50 microns of a TG1-1 tumor in the dorsal skinfold chamber of a TIE2-GFP mouse. The pre-application average relative signal was 0.349 ± 0.025 while the post-application average signal was 0.127 ± 0.017 , a statistically significant ($p < 0.001$) decrease.



We plan on evaluating each of our techniques *in vitro* first before attempting them *in vivo* in the living tumor, so I expect similar pilot studies on upcoming Tasks to occur over the next year or two, leading up to their commencement *in vivo* on the schedule dictated by the original Statement of Work.

The rich collaborative environment at URM C has produced an intriguing possibility for an alternative method to image the calcium fluctuations in tumor endothelial cells *in vivo*, which forms the cornerstone of the first 6 aims of this grant (those 6 aims are scheduled to commence in years 3 or later). In collaboration with Ian Dickerson and Anne Leubke in the Department of Neurobiology and Anatomy and Biomedical Engineering, respectively, we are testing polarization-sensitive multiphoton Fluorescence Resonance Energy Transfer (FRET) as a way to quantify calcium in endothelial cells *in vivo* using genetic calcium indicators. Polarization FRET quantifies the interaction between two fluorophores via the loss in the polarization “memory” that occurs when signal is first transferred between fluorophores before emission, versus simple emission where polarization “memory” is better retained [4]. Depending upon the success of our preliminary experiments, we would implement this new method by

developing transgenic mice which express the FRET-based genetic calcium sensors known as “cameleons” [5], modified to utilize the polarization-FRET-friendly fluorophores Cerulean and Venus [4]. These mice would express the calcium reporter gene under control of the strong β -actin promoter, which would be silenced except in cells which express TIE-2, namely the endothelial cells. However, we must first determine if we can quantify polarization FRET on our multiphoton laser-scanning microscope. To do this we transiently transfected NIH3T3 cells with a preexisting Cerulean-Venus FRET construct as well as a negative control expressing Cerulean and Venus separately. As shown in figure 6a and b, we successfully demonstrated the expected loss in anisotropy (i.e. the polarization ‘memory’) generated when FRET occurs. Next we will investigate this system’s sensitivity to scattering by introducing different concentrations of intralipid on top of the cells. This will allow us to determine if polarization FRET is sufficiently robust to be used *in vivo*, and over what imaging depths it is effective. If those tests are successful, we will test the specific calcium indicator *in vitro*, and if those tests in turn are successful, we will generate the requisite mice. This will allow us to avoid the tedious, noisy, and invasive method of directly adding AM-loaded calcium indicator to the surface of our tumors and rapidly accelerate our pursuit of our Tasks.

Figure 6a. Anisotropy images of NIH3T3 cells expressing a Cerulean-Venus FRET construct and imaged with a polarization-sensitive detection scheme on our MPLSM. When FRET occurs, the anisotropy is expected to be low, as is shown. Compare to figure 6b.

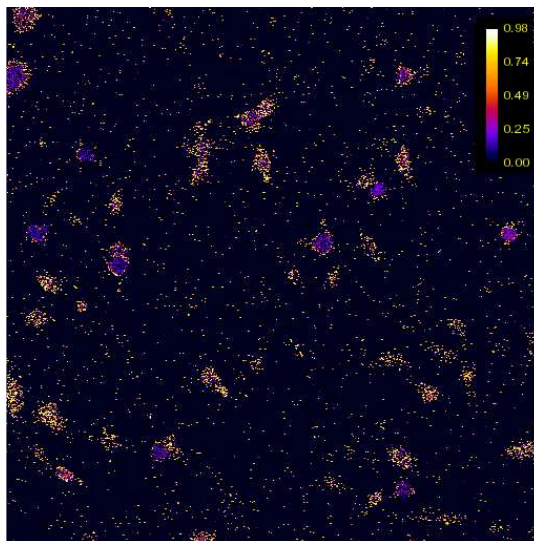
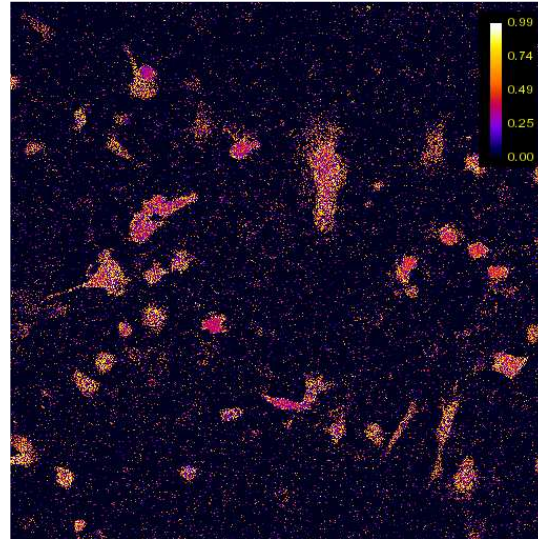


Figure 6b. Anisotropy images of NIH3T3 cells expressing a Cerulean-Venus construct in which FRET is not expected to occur. The anisotropy is therefore expected to be high, as is shown. Compare to figure 6a.



Key Research Accomplishments in the first 10 months:

Started a new laboratory, constructed our multiphoton laser-scanning microscope, and selectively recruited one junior faculty and three graduate students from a significantly larger pool of applicants.

Commenced work on Task 7a, the validation of the theory of MPFRAP with flow, by testing its ability to simultaneously measure diffusion and convection in situations where both are known.

Commenced work on Tasks 7b and c, the application of MPFRAP *in vivo* for the quantification of diffusion and flow in tumors and near tumor vessels.

Commenced work on Task 9c, the evaluation of second harmonic imaging of the local matrix as a reproducible measure of photodamage during permeability measurements.

Began preliminary work on Tasks 1-6, destined to start in 2-3 years, utilizing the MPLSM and *in vivo* calcium imaging to investigate the internal calcium dynamics of tumor endothelial cells.

Investigated a promising alternative to measuring calcium with AM-loaded indicator dyes, specifically polarization FRET imaging of genetic calcium indicators.

Reportable Outcomes:

Over the last ten months I have contributed to one manuscript:

Schmidt H, **Brown E**, Schwaller B, Eilers J. (2006) Parvalbumin is freely mobile in axons, somata, and nuclei of cerebellar Purkinje neurons. *Manuscript Submitted - will be furnished to DOD when published.*

I have also completed four book chapters:

Jain RK, Fukumura D, Munn L, **Brown E** (2006) Optical Microscopy in Small Animal Research. In: Tavitian B., Leroy-Willig A., Ntziachristos V. (ed) Imaging of Vertebrates. Wiley & Sons London, UK *In Press*

Jain RK, Fukumura D, Munn L, **Brown E** (2006) Tumor Angiogenesis and Blood Flow. In: Tavitian B., Leroy-Willig A., Ntziachristos V. (ed) Imaging of Vertebrates. Wiley & Sons London, UK *In Press*

Brown E, Majewska A, Jain RK (2006) Photobleaching and Recovery with Nonlinear Microscopy. In: So P., Masters B. (eds) Handbook of Biological Nonlinear Optical Microscopy. Oxford University Press, Oxford UK. *In Press*

Jain RK, Booth M, Padera T, Munn L, Fukumura D, **Brown E**. (2006) Applications of Nonlinear Intravital Microscopy in Tumor Biology. In: So P., Masters B. (eds) Handbook of Biological Nonlinear Optical Microscopy. Oxford University Press, Oxford UK. *In Press*

I have also given two invited talks:

“High Resolution Imaging of Tumor Biology and Treatment” Invited lecture presented at the Engineering Foundation’s Advances in Optics for Biotechnology, Medicine, and Surgery, Breckenridge, CO, 2005

“Nonlinear Microscopy of Living Tumors and Their Treatment” Invited lecture presented at the IEEE International Symposium on Biomedical Imaging, Arlington, VA, 2006

I have also applied for four grants while supported by this award:

“In vivo quantification of matrix metalloproteinase inhibitor efficacy”

Role: Principal Investigator

Effort: 20%

Agency: Department of Defense BCRP

Type: Idea Award BC060588

Period: 9/1/07-8/31/10

The goal of this study is to utilize novel microscopy techniques to explore the basic biology of MMP/collagen interactions in breast tumor models *in vivo*.

“The influence of neuronal activity on breast tumor metastasis to the brain”

Role: co-Investigator

Effort: 20%

Agency: Department of Defense BCRP

Type: Synergistic Idea Award

Period: 3/1/07-2/31/09

The goal of this study is to utilize insights gained from the study of dendritic spine motility to discover novel reagents that inhibit breast tumor metastases in the brain.

“*In vivo* assessment of MMPI efficacy”

Role: Principal Investigator

Effort: 4%

Agency: Johnson and Johnson Discovery Fund

Period: 9/1/06-8/31/07

The goal of this study is to explore novel methods to quantify MMP inhibitor efficacy in breast tumor models *in vivo*.

“In vivo quantification of MMP inhibitor efficacy using second harmonic generation microscopy”

Role: Principal Investigator

Effort: 20%

Agency: Department of Defense BCRP

Type: Concept Award BC051916

Period: 9/1/06-8/31/07

The goal of this study was to explore novel methods to quantify MMP inhibitor efficacy in breast tumor models *in vivo* using second harmonic generation. It was not awarded.

I feel that the content of these grant applications are noteworthy in that they show that the Era of hope Scholar Award has produced a long-term commitment to breast cancer research in my laboratory and has allowed me to attract other scientists (i.e. Dr. Majewska of the Department of Neurobiology and Anatomy, P.I. of the aforementioned Synergistic Idea Award application) to breast cancer research.

Conclusion

In conclusion, I believe that I have made significant progress on the goals outlined in my Era of Hope Scholar Award. In spite of starting the grant in September while simultaneously setting up a new laboratory and recruiting new personnel, I am fully on schedule in the two Tasks that were intended to commence in the first year (and be completed at the end of the second year), and I have even made progress in several Tasks that were not scheduled to start until next year or later. The most substantive result to date has been the beginning of the validation of equation (1) by direct testing against a gold standard *in vitro*. In addition, we have begun to evaluate MPFRAP *in vivo*, and begun to investigate measures of photodamage as described in Task 9. The significance of this work is that I am on schedule and have laid the strong foundation required for completion of the upcoming Tasks in my overall research plan.

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2. Brown, E., et al., *Dynamic imaging of collagen and its modulation in tumors in vivo using second harmonic generation*. Nat Med, 2003. **9**(6): p. 796-801.
3. Williams, R.M., W.R. Zipfel, and W.W. Webb, *Interpreting second-harmonic generation images of collagen I fibrils*. Biophys J, 2005. **88**(2): p. 1377-86.
4. Rizzo, M.A. and D.W. Piston, *High-contrast imaging of fluorescent protein FRET by fluorescence polarization microscopy*. Biophys J, 2005. **88**(2): p. L14-6.
5. Miyawaki, A., et al., *Fluorescent indicators for Ca²⁺ based on green fluorescent proteins and calmodulin*. Nature, 1997. **388**(6645): p. 882-7.

Curriculum Vitae

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Educational Background:

BS Magna Cum Laude with Honors in Physics, Wake Forest University 1992

MS Physics, Cornell University 1995

PhD Physics, Cornell University 1999

Professional Positions:

Doctoral Training

Graduate Student, Department of Physics, Cornell University
Laboratory of Dr. Watt Webb June 1992-July 1999

Post-Doctoral Fellow, Department of Radiation Oncology, Harvard Medical School
Massachusetts General Hospital
Laboratory of Dr. Rakesh Jain July 1999 – December 2001
Recipient of NRSA F32 Postdoctoral Research Award July 2000 - June 2002

Academic Positions

Instructor, Department of Radiation Oncology, Harvard Medical School
Massachusetts General Hospital January 2002-June 2005
Recipient of Whitaker Biomedical Engineering Research Grant July 2003
Recipient of American Cancer Society Institutional Research Grant July 2003
Assistant Professor, Department of Biomedical Engineering, University of Rochester
Medical Center September 2005-
Recipient of Department of Defense Era of Hope Scholar Award September 2005

Professional Memberships:

Biophysical Society	1995 -
American Association for Cancer Research	1999 -

Awards and Honors:

National Merit Scholarship	1988
Reynolds Scholarship - Wake Forest University (full tuition/room/board, stipend)	1988 - 1992
Phi Beta Kappa	1992
Omicron Delta Kappa, National Service Honor Society	1992
President, Sigma Pi Sigma WFU chapter National Physics Honor Society	1992
President, Alpha Phi Omega WFU chapter National Service Fraternity	1992
Speas Award for Excellence in Physics	1992
AFLAC-AACR Scholars in Cancer Research Award	2000
NRSA F32 Postdoctoral Research Award	2000 - 2002
Whitaker Biomedical Engineering Research Grant	2003 -
American Cancer Society Institutional Research Grant	2003
Department of Defense Era of Hope Scholar Award	2005 -

National Service Responsibilities:

Scientist Reviewer - Grant Review Panel, Department of Defense
Breast Cancer Research Program, Vienna, VA, 2002, 2004
Scientist Reviewer - Hypertension and Microcirculation NIH Study Section, 2004
Scientist Reviewer - UPMC Johnson and Johnson Discovery Concept Fund, 2005
Advisory Board - Nebraska Center for Cell Biology, 2004-
Served as reviewer for Nature Methods, Applied Optics, Optics Express, Journal of
Biomedical Optics, Cancer Research, Microvascular Research,
Microcirculation, Photochemistry and Photobiology.

Teaching Experience:

Teaching Assistant, Cornell University Department of Physics Physics 213 Electricity and Magnetism Physics 214 Waves and Optics	1992, 1993
Instructor, Cold Spring Harbor Laboratory summer course Four courses entitled "Imaging Structure and Function in the Nervous System" Lectured on and demonstrated the use of the Multiphoton Laser Scanning Microscope Course Organizer - "Methods in Biomedical Engineering"	1995 - 1998 2001 - 2003
Organized Steele Laboratory course, designed syllabus, lectured on light microscopy, fluorescence photobleaching recovery, and multiphoton microscopy	

Lecturer, MIT Chemical Engineering, Course 10.548J 2003
Course entitled "Tumor Pathophysiology and Transport Phenomena"
Lectured on *in vivo* multiphoton microscopy

Lecturer, University of Rochester Institute of Optics, Optics 476 2005
Course entitled "Biomedical Optics"
Lectured on *in vivo* multiphoton microscopy

Lecturer, University of Rochester Institute of Optics Summer Courses 2005-
Courses entitled "Biomedical Optics" and "High-Resolution Microscopy"
Lectured on epifluorescence, confocal, and multiphoton microscopy

Student Supervision

"Fluorescence Photobleaching Recovery with Spatial Fourier Analysis" 2000-01
One undergraduate worked for two consecutive summers, a second undergraduate worked for two consecutive summers and the intervening year in which he received credit through the MIT Undergraduate Research Opportunities Program

"Multiphoton Fluorescence Photobleaching Recovery with Flow" 2004
Undergraduate worked for a summer in which she received credit through the MIT Undergraduate Research Opportunities Program

"Calcium Signaling in Tumor Endothelial Cells" 2004
Medical student worked for a summer through my Whitaker Biomedical Engineering Research grant.

Graduate Student Supervision

Provided direct supervision for three graduate students (two M.I.T. Ph.D. candidates and one Harvard Medical School M.D.-Ph.D. candidate)
1999-2005

Currently supervising one University of Rochester Ph.D. candidate from the Department of Physics, one from the Institute of Optics, and one from the Biomedical Engineering Department
2005-

Publications:

Peer-Reviewed Journals:

Shear J, **Brown E**, Webb W. (1996) Multiphoton-excited fluorescence of fluorogen-labeled neurotransmitters. *Anal Chem* 68(10) 1778-1783.

Brown E, Shear J, Adams S, Tsien R, Webb W. (1999) Photolysis of caged calcium in femtoliter volumes using two-photon excitation. *Biophysical Journal* 76, 489-499.

- Brown E**, Wu E-S, Zipfel W, Webb W. (1999) Measurement of molecular diffusion in solution by multiphoton fluorescence photobleaching recovery. *Biophysical Journal* 77, 2837-2849.
- Majewska A, **Brown E**, Ross J, Yuste R. (2000) Mechanisms of calcium decay kinetics in hippocampal spines: role of spine calcium pumps and calcium diffusion through the spine neck in biochemical compartmentalization. *J Neuroscience* 20(5):1722-1734.
- Pluen A, Boucher Y, Ramanujan S, McKee TD, Gohongi T, di Tomaso E, **Brown E**, Izumi Y, Campbell R, Berk D, Jain RK. (2001) Role of tumor-host interactions in interstitial diffusion of macromolecules: cranial vs. subcutaneous tumors. *Proc Natl Acad Sci.* Apr 10;98(8):4628-33.
- Brown E**, Campbell R, Tsuzuki Y, Xu L, Carmeliet P, Fukumura D, Jain RK. (2001) *In vivo* measurement of gene expression, angiogenesis, and physiological function in tumors using multiphoton laser scanning microscopy. *Nature Medicine* 7(7): 864-868.
- Migliorini C, Qian Y, Chen H, **Brown E**, Jain RK, Munn L. (2002) Red blood cells augment leukocyte rolling in a virtual blood vessel. *Biophysical Journal* 83, 1834-41.
- Ramanujan S, Pluen A, McKee T, **Brown E**, Boucher Y, Jain RK. (2002) Diffusion and convection in collagen gels: relation to transport hindrance in the tumor. *Biophysical Journal* 83, 1650-1660.
- Padera T, Kadambi A, di Tomaso E, Mouta-Carreira C, **Brown E**, Boucher Y, Choi N, Mathisen D, Wain J, Mark E, Munn L, Jain RK. (2002) Lymphatic metastasis in the absence of functional intratumor lymphatics. *Science* Jun 7;296(5574):1883-6.
- Campbell R, Fukumura D, **Brown E**, Mazzola L, Izumi Y, Jain RK, Torchilin V, Munn L. (2002) Cationic charge determines the distribution of liposomes between the vascular and extravascular compartments of tumors. *Cancer Research* 62(23):6831-6.
- Wei X, Henke V, Strübing C, **Brown E**, Clapham D. (2003) Real time imaging of nuclear permeation by EGFP in single intact cells. *Biophysical Journal* 84:1317-1327.
- Schmidt H, **Brown E**, Schwaller B, Eilers J. (2003) Diffusional mobility of parvalbumin in spiny dendrites of cerebellar Purkinje neurons quantified by fluorescence recovery after photobleaching *Biophysical Journal* 84:2599-2608.

- Brown E***, McKee T*, di Tomaso E, Pluen A, Seed B, Boucher Y, Jain RK. (2003) Dynamic imaging of collagen and its modulation in tumors *in vivo* using second harmonic generation. *Nature Medicine* 9(6):796-801 *contributed equally.
- Abdul-Karim A, Al-Kofahi K, **Brown E**, Jain RK, Roysam B. (2003) Automated tracing and change analysis of angiogenic vasculature from *in vivo* multiphoton image time series. *Microvascular Research* 66(2):113-125
- Znati C, Rosenstein M, McKee T, **Brown E**, Turner D, Bloomer WD, Watkins S, Jain RK, Boucher Y. (2003) Irradiation Reduces Interstitial Fluid Transport and Increases the Collagen Content in Tumors. *Clin Cancer Res* 9:5508-5513
- Alexandrakis G, **Brown E**, Tong R, McKee T, Campbell R, Boucher Y, Jain RK. (2004) Two-photon fluorescence correlation microscopy to quantify the transport of fluorescently labeled tracers in tumors. *Nature Medicine* 10(2):203-207.
- Garkavtsev I, Kozin S, Chernova O, Xu L, **Brown E**, Barnett G, Jain RK. (2004) *ING4*: a novel regulator of brain tumor growth and angiogenesis. *Nature* 428:328-332.
- Brown E**, Boucher Y, Nasser S, Jain RK. (2004) Measurement of macromolecular diffusion coefficients in human tumors. *Microvascular Research* 67(3):231-236
- Duda D, Fukumura D, Munn L, Booth M, **Brown E**, Huang P, Seed B, Jain RK (2004) Differential transplantability of tumor-associated stromal cells *Cancer Research* 64: 5920-5924
- Stroh M, Zimmer J, Duda D, Levchenko T, Cohen K, **Brown E**, Scadden D, Torchilin V, Bawendi M, Fukumura D, and Jain RK. (2005) Quantum dots spectrally distinguish multiple species within the tumor milieu *in vivo*. *Nature Medicine* 11(6):678-682
- Tyrrell J, Mahadevan V, Tong R, **Brown E**, Jain RK, Roysam B. (2005) 3D model-based complexity analysis of tumor microvasculature from *in vivo* multiphoton Images. *Microvascular Research* 70(3):165-178
- Schmidt H, **Brown E**, Schwaller B, Eilers J. (2006) Parvalbumin is freely mobile in axons, somata, and nuclei of cerebellar Purkinje neurons. *Manuscript Submitted*.

Book chapters:

- Brown E**, Webb W. (1998) Two-Photon Activation of Caged Calcium with Submicron, Submillisecond Resolution. In Mariott G (ed). Caged Compounds. Methods of Enzymology series v. 291 Academic Press, San Diego. pp. 356-380

- Jain RK, **Brown E**, Munn L, Fukumura D. (2004) Intravital Microscopy of Normal and Diseased Tissue in the Mouse. In: Spector D, Goldman R. (eds). Live Cell Imaging: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. Chapter 24, pp 435-466.
- Brown E**, Majewska A, Jain RK. (2005) FRAP and Multiphoton FRAP. In: Yuste R, Konnerth A. (eds). Imaging in Neuroscience and Development: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. Chapter 56, pp. 429-438.
- Brown E**, Munn L, Fukumura D, Jain RK.(2005) A Practical Guide to *in vivo* Imaging of Tumors. In: Yuste R, Konnerth A. (eds). Imaging in Neuroscience and Development: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. Chapter 92, pp. 695-700.
- Jain RK, Fukumura D, Munn L, **Brown E** (2006) Optical Microscopy in Small Animal Research. In: Tavittian B., Leroy-Willig A., Ntziachristos V. (ed) Imaging of Vertebrates. Wiley & Sons London, UK *In Press*
- Jain RK, Fukumura D, Munn L, **Brown E** (2006) Tumor Angiogenesis and Blood Flow. In: Tavittian B., Leroy-Willig A., Ntziachristos V. (ed) Imaging of Vertebrates. Wiley & Sons London, UK *In Press*
- Brown E**, Majewska A, Jain RK (2006) Photobleaching and Recovery with Nonlinear Microscopy. In: So P., Masters B. (eds) Handbook of Biological Nonlinear Optical Microscopy. Oxford University Press, Oxford UK. *In Press*
- Jain RK, Booth M, Padera T, Munn L, Fukumura D, **Brown E**. (2006) Applications of Nonlinear Intravital Microscopy in Tumor Biology. In: So P., Masters B. (eds) Handbook of Biological Nonlinear Optical Microscopy. Oxford University Press, Oxford UK. *In Press*

Funding History

Ongoing Research Support:

"Angiogenic Signaling in Living Breast Tumor Models"

Role: Principal Investigator

Agency: Department of Defense BCRP

Type: Era of Hope Scholar Award W81XWH-05-1-0396

Period: 09/01/05-08/31/10.

The goal of this study is to investigate the signaling mechanism relating VEGFR2 activation to tumor vessel permeability, and to further develop the relevant noninvasive optical techniques.

"Imaging Angiogenesis and Vascular Function in Tumors"

Role: Principal Investigator

Agency: The Whitaker Foundation

Type: Biomedical Engineering Research Grant RG-05-0007

Period: 08/01/03-07/31/06

The goal of this study is to investigate dynamic properties of host-derived VEGF-expressing perivascular cells and to determine their influence on the physiological function of adjacent tumor blood vessels.

Completed Research Support:

Dates	Grant Number	P.I.	Type
1996-1999	NIH#T32-GM08267	Dr. Watt Webb	Training Grant
1999-2000	NIH#T32-CA73479	Dr. Rakesh Jain	Training Grant
2000-2002	NIH#F32-CA88490	Dr. Ed Brown	NRSA Postdoctoral Award
2000-2005	NIH#P01 CA80124	Dr. Rakesh Jain	NIH Program Project Grant
2000-2005	NIH#R24 CA85140	Dr. Rakesh Jain	BRP
2003-2004	IRG-87-007-13	Dr. Ed Brown	American Cancer Society

Invited Lectures

"3D-Resolved Uncaging of Calcium Using Two-Photon Excitation." Invited lecture presented at Max Planck Institut fur Biophysikalische Chemie. Gottingen, Germany, 1997.

"Uncaging Using Two-Photon Microscopy." Invited lecture presented at Universitat des Saarlandes, Homburg, Germany, 1997.

"Quantitative Photochemical Applications of Multiphoton Laser-Scanning Microscopy." Invited lecture presented at Wake Forest University Dept. of Physics, Winston-Salem, NC, 1999.

"Quantitative Photochemical Applications of Multiphoton Laser-Scanning Microscopy." Invited lecture presented at Harvard University Dept. of Physics, Cambridge, MA, 1999.

"Quantitative Photochemical Applications of Multiphoton Laser Scanning Microscopy." and "In Vivo Measurement of Tumor Angiogenesis, Gene Expression, and Physiological Function Using the MPLSM" Invited lectures presented at Wellman Laboratory, MGH, Boston, MA, 2000.

"Measurement of physiological parameters in tumors in vivo using MPLSM." Invited lecture presented at the SPIE meeting on Progress in Biomedical Optics and Imaging, San Jose, CA, 2001. Published as: Brown E, Campbell R, Tsuzuki Y, Fukumura D, Jain RK. Measurement of physiological parameters in tumors in vivo using MPLSM. In: Periasamy A, So PTC, (eds). Progress in Biomedical Optics and Imaging. Proceedings of SPIE vol. 4262 Jan 21-23, Bellingham, Washington. SPIE; 2001 p. 134-146.

"Molecular Imaging of VEGF and HIF1 alpha." Invited lecture presented at the RCCA symposium, Dana-Farber Cancer Institute, Boston, MA, 2001.

"Quantitative Photochemical Applications of Multiphoton Laser-Scanning Microscopy." Invited lecture presented at the Renal Unit, MGH, Boston, MA, 2001.

"Microscopy of Living Tumors" Invited lecture presented at the CenSSIS Research and Industrial Collaboration Conference, Northeastern University, Boston, MA, 2002.

"Nonlinear Optical Microscopy of Tumors" Invited lecture presented at the Northeast Proton Center, Boston, MA, 2002.

“Dynamic Imaging of Collagen Content and Structure in Tumors Using Second Harmonic Generation.” Short Talk presented at the SPIE meeting on Progress in Biomedical Optics and Imaging, San Jose, CA, 2003.

“Dynamic Imaging of Collagen in Tumors *in vivo* Using Second Harmonic Generation.” Short Talk presented at the Keystone Symposium on Optical Imaging: Applications to Biology and Medicine, Taos, NM, 2003.

"Multiphoton Imaging of Tumor Biology and Angiogenesis" Invited lecture presented at the Department of Molecular Therapeutics, MD Anderson Cancer Center, Houston, TX, 2003

"Two Photon Imaging of Tumors" Invited lecture presented at the Department of Anatomy and Structural Biology, Albert Einstein College of Medicine, Bronx, NY, 2003

"Two Photon Imaging of Tumors" Invited lecture presented at the Department of Cell Biology, Yale University School of Medicine, New Haven, CT, 2003

"Two Photon Imaging of Tumors" Invited lecture presented at the Bioengineering Division, University of California-San Francisco, San Francisco, CA, 2003

"Two Photon Imaging of Tumors" Invited lecture presented at the Department of Radiation Oncology, University of Cincinnati, Cincinnati, OH, 2003

"Two Photon Imaging of Tumors" Invited lecture presented at the Department of Radiation Oncology, University of Michigan, Ann Arbor, MI, 2003

"Two Photon Imaging of Tumors" Invited lecture presented at the Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, Chapel Hill, NC, 2003

"Two Photon Imaging of Tumors" Invited lecture presented at the Department of Radiology, University of Utah, Salt Lake City, UT 2003

"Tumor Pathophysiology Studied with the Multiphoton Laser-Scanning Microscope" Invited lecture presented at the Microscopy and Microanalysis Symposium: Advances in Quantitative Optical Microscopy, a Symposium in Honor of Watt Webb. San Antonio, TX, 2003.

"Diverse Imaging Processing Challenges in Radiation Oncology" Invited lecture presented at the Image Processing Mini-workshop, Massachusetts General Hospital, Boston, MA, 2004

"Multiphoton Imaging of Tumor Biology and Angiogenesis" Invited lecture presented at the Interdepartmental Program in Vascular Biology and Transplantation, Yale Medical School, New Haven, CT, 2004

"Can Antitumor Therapy be Improved with Multiphoton Laser-Scanning Microscopy?" Invited lecture presented at the Department of Biomedical Engineering, Yale University, New Haven, CT, 2004

"Nonlinear Optical Microscopy of Tumors" Invited lecture presented at the Biomedical Optical Spectroscopy, Imaging & Diagnostics Topical Meeting of the Optical Society of America. Miami, FL, 2004

"Can Antitumor Therapy be Improved with Multiphoton Laser-Scanning Microscopy?" Invited lecture presented at the Department of Cancer Biology, Vanderbilt University, Nashville, TN, 2004

"Can Antitumor Therapy be Improved with Multiphoton Laser-Scanning Microscopy?" Invited lecture presented at the International Institute of Molecular and Cellular Biology, Warsaw, Poland, 2004

"Can Antitumor Therapy be Improved with Multiphoton Laser-Scanning Microscopy?"
Invited lecture presented at the Department of Bioengineering, PAN, Warsaw, Poland, 2004

"Can Antitumor Therapy be Improved with Multiphoton Laser-Scanning Microscopy?"
Invited lecture presented at the Department of Biomedical Engineering, Cornell University, Ithaca, NY, 2004

"Multiphoton Imaging of Tumor Biology and Angiogenesis." Invited lecture presented at the Department of Biomedical Engineering, University of Rochester Medical Center, Rochester, NY, 2004

"Measurement of Diffusion Coefficients *in Vivo*: Why and How?" Invited lecture presented at the Department of Physics, Creighton University, Omaha, Nebraska, 2004

"Tumor Photobiology" Invited lecture presented at the First Annual Symposium on Modern Imaging and Biophysical Methods in Cell Biology and Neuroscience. Creighton University, Omaha, Nebraska, 2004

"Multiphoton Imaging of Tumor Pathophysiology" Invited lecture presented at the Center for Engineering in Medicine, Shriners Hospitals for Children, Boston, MA, 2004

"High Resolution Imaging of Tumor Biology and Treatment" Invited lecture presented at the Engineering Foundation's Advances in Optics for Biotechnology, Medicine, and Surgery, Breckenridge, CO, 2005

"Nonlinear Microscopy of Living Tumors and Their Treatment" Invited lecture to be presented at the IEEE International Symposium on Biomedical Imaging, Arlington, VA, 2006

Selected Abstracts

Shear J, **Brown E**, Adams S, Tsien R, Webb W. Two-photon excited photorelease of caged calcium. Annual Meeting of the Biophysical Society. 1996.

Brown E, Ellis-Davies G, Webb W. Quantitative two-photon calcium uncaging. Annual Meeting of the Biophysical Society 1998.

Majewska A, **Brown E**, Yuste R. Mechanism of calcium decay kinetics in spines from hippocampal pyramidal neurons. Society for Neuroscience Abstracts, 1999.

Brown E, Pluen A, Compton C, Boucher Y, Jain RK. Measurement of diffusion coefficients of spontaneous human tumors. Annual Meeting of the American Association for Cancer Research, 2000.

Padera T, Kadambi A, Stoll B, di Tomaso E, Mouta-Carreira C, **Brown E**, Munn L, Jain RK. Multiphoton imaging of VEGF-C induced peri-tumor lymphatic hyperplasia. Gordon Research Conference on Angiogenesis and Microcirculation, 2001.

Brown E, Boucher Y, Jain RK. Measurement of diffusion coefficients in spontaneous human tumors. Annual Meeting of the American Association for Cancer Research, 2002.

Campbell R, **Brown E**, Izumi Y, Mazzola L, Torchilin V, Fukumura D, Munn L, Jain RK. Interactions of PEGylated cationic liposomes with tumor vessels in mice. Annual Meeting of the American Association for Cancer Research, 2002.

Brown E, Fukumura D, Munn L, Jain RK. Imaging the motion of host cells in tumors *in vivo* using intravital microscopy. Annual Meeting of the Molecular Imaging Society 2002.

- Abdul-Karim M, Al-Kofahi O, **Brown E**, Jain RK, Al-Kofahi K, Turner J, Roysam B. Automated *in vivo* change analysis of tumor vasculature from two-photon confocal image time series. SPIE meeting on Progress in Biomedical Optics and Imaging, 2003.
- Brown E**, McKee T, Pluen A, Boucher Y, Jain RK. Dynamic imaging of collagen content and structure in tumors using second harmonic generation. International Society for Optical Engineering Meeting on Progress in Biomedical Optics and Imaging, 2003.
- Brown E**, McKee T, Pluen A, Boucher Y, Jain RK. Dynamic imaging of collagen in tumors *in vivo* using second harmonic generation. Keystone Symposium on Optical Imaging: Applications to Biology and Medicine, 2003.